The effects of spinal NMDA receptor activation on KCC2, EphB2 and n-cadherin and its implications for hyperalgesic priming

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Abstract

N-Methyl-D-aspartate glutamate receptor (NMDAR) function is crucial to synaptic plasticity in the CNS, including nociceptive sensitization in the pain pathway. One way that NMDAR function affects synaptic function is by interacting with membrane proteins such as potassium-chloride cotransporter 2 (KCC2) and the ephrine B2 receptor (EphB2R) (Song et al). Recent studies show that NMDAR activation or peripheral nerve injury leads to a µ-opioid-mediated decrease in KCC2 expression (Zhou et al). Additionally, blockade of spinal NMDAR was shown to prevent BDNF-induced thermal sensitivity in naïve rats. While these data clearly indicate that this activity is important to pain processing in the spinal cord, the coincidence of KCC2 and EphB2R expression in this processing has not been previously investigated. In our study, we hypothesized that NMDAR activation can lead to concurrent changes of KCC2 and EphB2R expression, both of which are expressed in excitatory synapses. Using spinal synaptoneurosomes (SNS) from mice, we demonstrated that NMDAR activation with 300µM NMDA led to a decrease in the monomeric form of KCC2 and EphB2R within 15 min. These effects were completely blocked by co-incubation of NMDA with physiological levels of Mg2+ (2mM). Subsequently, we tested the effects of in vivo activation of NMDA by injecting brain-derived neurotrophic factor (BDNF) into the spinal cord. We showed that BDNF-induced allodynia is prevented by the NMDAR blocker D-AP5, demonstrating that BDNF requires NMDAR activation for the establishment of mechanosensitive allodynia. Additionally, at 24 hours after BDNF injection, we observed reduced expression of EphB2R but not KCC2. Interestingly, even 7 days after BDNF injection, EphB2R expression remains altered. Furthermore, our investigation has expanded beyond the behavior of NMDAR-modulated KCC2 and EphB2R expression into exploration of additional interactions with n-cadherin, an important synaptic adhesion molecule. During NMDA SNS experiments, we observed that full-length n-cadherin decreased simultaneously with monomeric KCC2 and full-length EphB2R. We also saw that n-cadherin expression decreased 24 hours after BDNF injection but returned to baseline by Day 7 after injection. Our discovery suggests that NMDAR-mediated pro-nociceptive changes in synaptic spines may have profound implications in the initiation and persistence of pain.

Conclusions and Future Directions

1. Acute NMDA exposure causes downregulation of KCC2, EphB2R and n-cadherin in the spinal cord synaptoneurosomes.

2. BDNF-mediated NMDAR activation acutely reduces EphB2R and n-cadherin expression in the mouse dorsal spinal horn. EphB2R expression was found unchanged even 7 days after BDNF injection. Our lab has previously demonstrated that spinal cord plasticity is an important component of hyperalgesic priming, which is a model of acute to chronic transition of pain (Asiedu et al). Our current finding suggests that the expression of certain pro-nociceptive proteins such as EphB2R change with intrathecal injection of BDNF, which establishes hyperalgesic priming. Such changes seem to be mediated by NMDAR activation as the blockade of this receptor prevented the effects of BDNF. We plan to further investigate whether upregulation of EphB2R can serve as a marker for hypergesic priming by looking at EphB2R expression using other priming agents (i.e., IL-6 and carrageenan).

Materials and Methods

Synaptoneurosomes Preparation and Treatment

Spinal cord synaptoneurosomes (SNS) were isolated from 3-weeks-old (11-13g) male ICR mice. The collected tissues were homogenized in an artificial cerebrospinal fluid (aCSF) containing NaCl, KCl, MgSO4, CaCl2, KH2PO4 and glucose. Samples were filtered through 100μm and 1 μm nylon mesh filters (Millipore) and centrifuged at 10,000 x g for 20 min. The pellet was resuspended in the homogenization buffer without MgSO4 containing 300μM NMDA (Toxic) for 15 min at 37°C. The cellular fraction was collected by centrifugation at 20,000 x g for 2 min. The pellet was resuspended in lysis buffer (50 mM Tris HCl, 1% Triton X-100, 150 mM NaCl, and 1 mM EDTA at pH 7.4), ultracentrifuged and centrifuged at 200,000 x g for 10 min. The supernatant was collected and assayed using Western blot analysis.

Behavioral Testing

Male ICR mice were placed in acrylic boxes with wire mesh floors, and baseline mechanical withdrawal thresholds of the left hind paw were measured after habituation for 1 h using the up-down method (Chaplan et al., 1994). The experimenter making measurements was always blinded to the experimental conditions. 0.1ng BDNF was injected intrathecally into the spinal cord in a volume of 5μl. For D-AP5 experiment, the drug was injected 15 minutes prior to the BDNF intrathecal injection.

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References

